Annals of Botany **97**: 649–658, 2006
doi:10.1093/aob/mcl020, available online at www.aob.oxfordjournals.org

Cessation of Tillering in Spring Wheat in Relation to Light Interception and Red : Far-red Ratio

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Received: 17 October 2005 Returned for revision: 10 November 2005 Accepted: 22 December 2005 Published electronically: 7 February 2006

• *Background and Aims* The production of axillary shoots (tillering) in spring wheat (*Triticum aestivum*) depends on intraspecific competition. The mechanisms that underlie this competition are complex, but light within the wheat canopy plays a key role. The main objectives of this paper are to analyse the effects of plant population density and shade on tillering dynamics of spring wheat, to assess the canopy conditions quantitatively at the time of tillering cessation, and to analyse the relationship between the tiller bud and the leaf on the same phytomer.
• *Methods* Spring wheat plants were grown at three plant population densities and under two light regimes (25 % and 100 % light). Tiller appearance, fraction of the light intercepted, and red : far-red ratio at soil level were recorded. On six sampling dates the growth status of axillary buds was analysed.
• *Key Results* Tillering ceased earlier at high population densities and ceased earlier in the shade than in full sunlight. At cessation of tillering, both the fraction of light intercepted and the red : far-red ratio at soil level were similar in all treatments. Leaves on the same phytomer of buds that grew out showed more leaf mass per unit area than those on the same phytomer of buds that remained dormant.
• *Conclusions* Tillering ceases at specific light conditions within the wheat canopy, independent of population density, and to a lesser extent independent of light intensity. It is suggested that cessation of tillering is induced when the fraction of PAR intercepted by the canopy exceeds a specific threshold (0·40–0·45) and red : far-red ratio drops below 0·35–0·40.

Key words: *Triticum aestivum*, wheat, tiller, bud, plant population density, shade, PAR, red : far-red ratio, functional–structural model.

INTRODUCTION

Like most gramineous species, wheat (*Triticum aestivum*) produces tillers, which develop from axillary buds on the parent shoot, located at the bottom of the internode of the parent phytomer, just above the node and the sheath insertion point of the preceding phytomer. Theoretically, the parent shoot can be any existing shoot on the plant. Tillers that grow from the main stem are called primary tillers and those from primary tillers are secondary tillers, etc. (Kirby and Appleyard, 1981). In practice, however, only a few tiller buds grow into a tiller, and only a proportion of these tillers survive to become ear-bearing shoots. The ultimate number of tillers is therefore a result of tiller appearance and tiller survival. The main factors that affect the appearance and survival of tillers in the Gramineae are nitrogen (N) availability (e.g. Davies, 1971) and plant population density (Darwinkel, 1978). Population density affects tillering cessation primarily via two mechanisms. By the first, tillering appears to cease earlier in the development of the plant the higher the population density, due to the severely reduced PAR (photosynthetically active radiation) intensity at the lower levels inside the canopy, especially near complete canopy closure (Simon and Lemaire, 1987). In this context, a relationship has been assumed between outgrowth of tiller buds, PAR intensity and LMA (leaf mass per unit leaf area) of the associated 'parent' leaves (i.e. the leaf on the same phytomer as the bud) (Bos, 1999). By the second mechanism, increase in population density is associated with a lower red : far-red ratio (R : FR) at the base of the individual plants because there is more surrounding vegetation that can scatter red and far-red light differentially (Holmes and Smith, 1977). A low R : FR ratio reduces the phytochrome photoequilibrium (Pr : Pfr) (Smith, 2000), and has been related to reduced tillering (Casal *et al.*, 1986; Barnes and Bugbee, 1991). Decline in R : FR has been shown to herald mutual shading, acting as an early warning signal for future competition (Ballaré *et al.*, 1987; Franklin and Whitelam, 2005).

Wheat plants, as for gramineous species in general, do show variable degrees of tillering. There are two patterns of cessation of tillering. In the first, all tiller outgrowth is arrested, usually at the time of formation of the terminal spikelet on the parent shoot apex (Baker and Gallagher, 1983; Gomez-Macpherson *et al.*, 1998), which coincides with stem elongation (Hay, 1999). The plants switch to the reproductive phase and axillary buds differentiate into floral structures. In the second pattern, only vegetative tiller buds are initiated, the plant has not become reproductive, but buds stop producing tillers. The current study deals with this second type of cessation of tillering during the vegetative phase.

The objectives of the current work were (*a*) to study the kinetics of tiller appearance and the probability of tiller occurrence in spring wheat at three population densities

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and two light intensities; (*b*) to analyse cessation of tiller appearance in relation to the light conditions inside the canopy (in terms of PAR intercepted and R : FR when tillering ceases); and (*c*) to establish a possible link between the time of cessation of tillering and the LMA of the parent leaves.

MATERIALS AND METHODS

Experimental set-up

The data analysed in this study are from an outdoor experiment conducted under natural climatic conditions in Wageningen, The Netherlands (51°58′N), from April to June 2004. Spring wheat plants (*Triticum aestivum* L., 'Minaret') were grown in containers (70 × 90 cm), holding a soil layer approx. 35 cm deep, enriched with fertilizer resulting in a mineral nitrogen content of 15 g m^{-2}. Diseases, pests and weeds were controlled by using appropriate biocides. The containers were arranged closely together to ensure canopy homogeneity, and surrounded by guard containers to prevent border effects. Seeds were sown at three population densities (100, 262 and 508 m^{-2}) in a regular square grid with 10·0, 6·2 and 4·4 cm distance between the plants, respectively. All seeds produced a plant. The containers were exposed to full light (control). A similar set-up was created in a tent constructed of shade material (XLF 17F, US Global Resources, Seattle, USA) that absorbed 75 % of the incoming light. The six different population density × light intensity combinations are denoted as D1c, D2c, D3c, D1s, D2s and D3s with the number indicating the population density (1 = 100, 2 = 262, 3 = 508 plants m^{-2}) and the letters 'c' or 's' indicating control or shade, respectively. Due to the open mesh structure of the shade material the tent had a negligible effect on temperature and humidity, and the inside of the tent had a negligible effect on the red : far-red ratio of the reflected light. Additional containers with plants at the same three population densities were used for the destructive measurements.

Data collection and analysis

Temperature was recorded every hour with shielded TempControl thermocouples (type T, TempControl Industrial Electronic Products, Voorburg, NL) and a Datataker DT600 datalogger (Datataker Data Loggers, Cambridgeshire, UK). Thermocouples were placed in the soil (6 cm deep; 'soil temperature') and in the canopy (20 cm above soil level; 'canopy temperature'). Thermal time was calculated from temperature data using a resolution of 1 h. For the first 2 weeks, soil temperature was used, since the apex was still below soil surface; canopy temperature was used thereafter. The start of thermal time accumulation was set at the appearance of the first leaf and the base temperature used was 0 °C.

Tiller appearance from the surrounding sheath tissues, leaf appearance and length of the appeared part of each leaf of ten plants per treatment (n = 60) were monitored every 3 or 4 d; this was done for main stem and all primary tillers [denoted according to the main stem phytomer number from which the tiller originated: t1 (the coleoptile tiller), t2, t3, etc.]. A leaf was considered to have appeared halfway between the last observation when it was absent and the first observation when the tip was visible. A tiller was considered to be senescing, when the youngest leaf stopped extending (Kirby and Riggs, 1978); this could be clearly discerned in the current experiment. The starting date of senescence was defined as halfway between the last observation when a tiller was not observed to be senescing and the first observation when it was judged to be senescing. To determine additional information on tillering kinetics, tiller appearance and senescence were recorded on tillers of all orders for ten extra plants per treatment, bringing the total number of plants for which records were taken to 120.

To express the mean developmental stage of the plants in a specific treatment, thermal time was converted into elapsed phyllochrons by fitting a second-order polynomial to the relationship between number of appeared leaves and thermal time. This was done to obtain an index of developmental stage (physiological age) that was not biased by the effect of treatment on phyllochron. Phyllochron is the most important determinant of rate of tillering due to the co-ordination of leaf and tiller appearance (Kirby *et al.*, 1985), and physiological age, expressed in phyllochrons, is a measure of plant development, comparable to the Haun stage (Haun, 1973). However, the Haun stage increases by one unit at leaf ligule appearance, whereas physiological age increases by one unit at appearance of the tip of a new leaf. Physiological age at maximum tiller number was studied in more detail by calculating it for individual plants for which exact leaf appearance kinetics were known (n = 60), and calculating means for each treatment. Similarly, tiller appearance was expressed using individual plant-based physiological age.

Bud growth of plants grown in natural light was assessed by harvesting three plants per population density on six sampling dates (at ligule appearance of leaves 3–8 on the main stem; n = 54). For each individual plant, the status of each main stem tiller bud that had not yet appeared was classed as either dormant or growing. A bud was considered to be dormant when the length of the prophyll was <7 mm. This threshold value was derived from the data in this investigation as larger prophylls were nearly all longer than 1 cm. This threshold value did not differentiate between dormant buds and growing buds that were still <7 mm in length of the prophyll, but the probability that the latter is the case is very small since buds grew rapidly after escaping from their cavity in the internode (Williams and Langer, 1975). Dimensions and dry weights of leaves on the parent phytomers of the buds examined were determined. Leaf mass per unit leaf area (LMA, in mg cm^{-2}) was calculated by dividing blade dry weight by blade area; the latter was obtained by calculating length × width × S. The value of the shape coefficient S was 0·81, obtained by integration of the blade shape function as explained in Appendix A. To examine the possible relationship between LMA of the leaf, and the bud on the same phytomer as the leaf, the bud development dataset was split into two groups,

TABLE 1. *The values of the fitted coefficients of eqns 1–3*

		D1c	D2c	D3c	D1s	D2s	D3s
P_{int} *vs.* t	$P_{int,m}$	1 (0*)	1 (0*)	1 (0*)	1 (0*)	0·91 (0·04)	0·92 (0·02)
(eqn 1)	t_m	445 (5·9)	355 (10·2)	328 (9·9)	394 (13·8)	238 (36·1)	171 (24·4)
	t_e	676 (11·8)	633 (12·0)	639 (11·4)	997 (25·3)	752 (83·0)	634 (24·7)
R *vs.* t	a	1·37 (0·06)	1·39 (0·06)	1·47 (0·06)	1·38 (0·04)	1·34 (0·04)	1·42 (0·06)
(eqn 2)	$b{\cdot}10^{-4}$	33·8 (2·9)	43·8 (3·8)	55·4 (4·4)	25·3 (1·8)	29·9 (1·7)	44·8 (3·4)
		Control			Shade		
R *vs.* P_{int}	c	0·87 (0·04)			1·27 (0·05)		
(eqn 3)	d	2·32 (0·19)			2·20 (0·12)		

The coefficients of eqn 1 were fitted to data on the fraction of PAR intercepted (P_{int}) versus thermal time. The coefficients of eqn 2 were fitted to R : FR (R) data versus thermal time. The coefficients of eqn 3 were fitted to R : FR (R) data versus fraction PAR intercepted.

Equations 1 and 2 were fitted for plots at three population densities (D1, D2 and D3, i.e. 100, 262 and 508 plants m^{-2}) and two light regimes (c = control, s = shade). Equation 3 was fitted for the two light regimes only (population densities combined).

In parentheses: ±s.e.

* The zero value for the standard error was a result of the fact that the coefficient was constrained in the fitting process as it was exceeding the imposed restriction ($P_{int,m} \leqslant 1$).

one group containing data from phytomers with buds that grew out ('outgrowth' group) and one group with buds that were dormant ('dormant' group).

Light penetration at approx. 2 cm above soil level was measured once every week around noon, at six randomly chosen locations in the microcanopy of each of the population density × light intensity combinations, using the SunScan Canopy Analysis System (Delta T Devices, Cambridge, UK). This device measures PAR along a light-sensitive bar. A separate reference sensor, which measured PAR input, was placed just above the canopy. The fraction of incoming PAR intercepted by the canopy, P_{int}, was fitted to thermal time using the beta growth function (Yin *et al*., 2003), which allows for asymmetry in the curve around the inflection point and does not have the x-axis for asymptote but originates from (0,0) (eqn 1):

$$P_{int} = P_{int,m}\left(1 + \frac{t_e - t}{t_e - t_m}\right)\left(\frac{t}{t_e}\right)^{\frac{t_e}{t_e - t_m}} \quad (1)$$

where P_{int} is the fraction of PAR intercepted, $P_{int,m}$ is the maximum fraction of PAR intercepted (which was restricted to $\leqslant$1 in the fitting process), t_e is the amount of elapsed thermal time at $P_{int,m}$, t_m is the amount of thermal time (°Cd) at the inflection point where the slope has its maximum value, and t is thermal time (°Cd) since the appearance of the first leaf.

Weekly measurements of red : far-red ratio (R : FR) at approx. 2 cm above soil level were made using the Skye SKR100/116 Fibre Optic Probe Measuring System (Skye Instruments Ltd, Powys, UK). Measurements were made parallel to the soil surface, with the sensor facing north. The device was equipped with a glass fibre probe that measured R : FR at its tip, with an angle of view of 40° relative to the soil surface. In each microcanopy, six measurements were made at random locations. R : FR was fitted to thermal time using a two-parameter exponential decline function, see eqn 2:

$$R = a\ \exp(-bt) \quad (2)$$

where R is red : far-red ratio, a and b are coefficients and t is thermal time (°Cd).

Equations 1 and 2 were used to convert thermal time to the fraction of PAR intercepted and R : FR respectively, in order to express canopy development and tillering kinetics as functions of these two measures of plant development. To fit the R : FR data to the fraction of PAR intercepted, a similar two-parameter exponential decline function was used, see eqn 3:

$$R = c\ \exp(-dP_{int}) \quad (3)$$

where R is red : far-red ratio, c and d are coefficients and P_{int} is the fraction of PAR intercepted.

Statistics

The difference between population densities on PAR intercepted and R : FR was tested using a t-test on the fitted coefficients. Statistical analyses of physiological age, PAR and R : FR at the time of maximum tiller number included analysis of variance and LSD *post hoc* tests. Non-linear fits were done using the NLIN procedure of The SAS System v8·02; further data analysis was done using SPSS v11·0.1 and Microsoft Excel 2003.

RESULTS

Fraction of PAR intercepted and red : far-red ratio

The increase in P_{int} with thermal time was very similar in D2c and D3c, because unconstrained coefficients t_m and t_e, describing the temporal change, did not differ significantly (Table 1 and Fig. 1A, B). This means that the thermal time of the steepest increase and maximum PAR intercepted were reached simultaneously. The course of PAR intercepted by D1c over thermal time was shifted to the right compared with those of D2c and D3c, as values of both coefficients for D1c were significantly higher (P < 0·001 for t_m and P < 0·05 for t_e) than those for D2c and D3c. For the shaded plants, the two coefficients describing the course of P_{int} over thermal time did not differ significantly between the treatments D2s and D3s, but coefficients of D1s differed from those of D2s and D3s (P < 0·05)

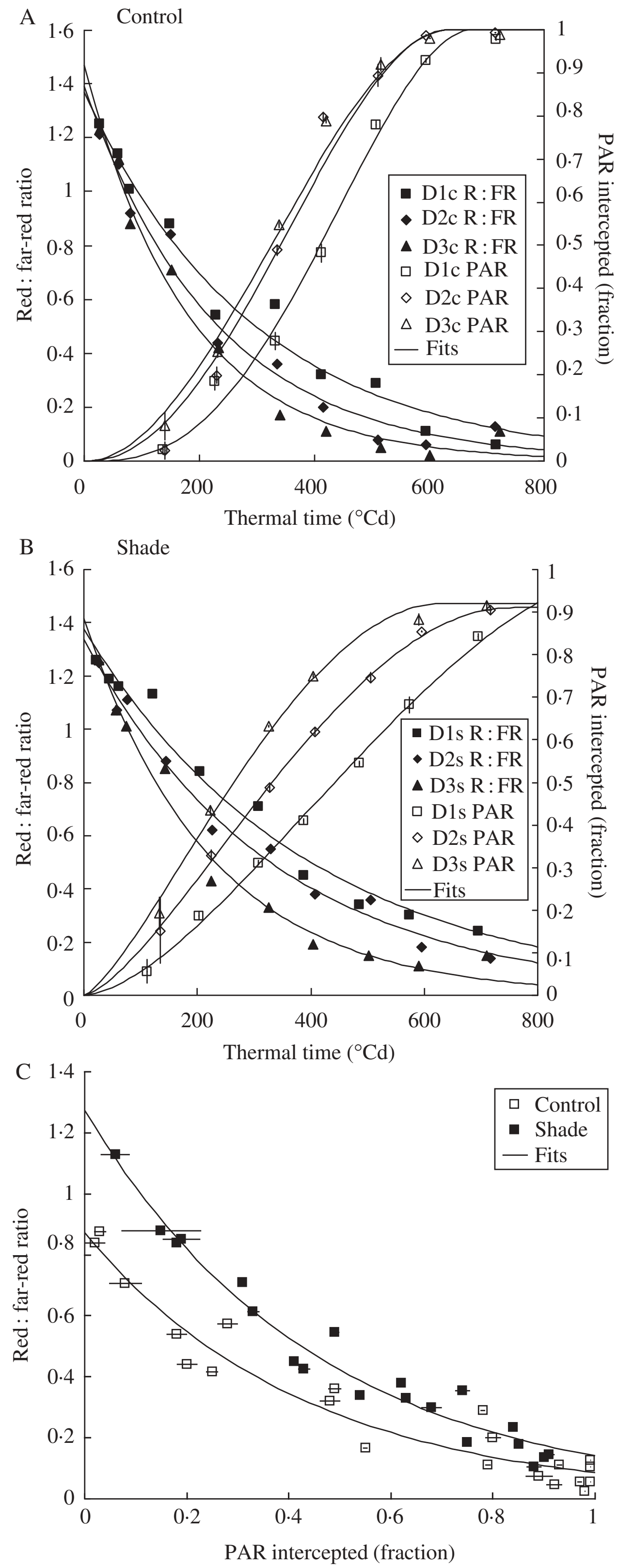

FIG. 1. Time courses of the fraction of PAR intercepted (open symbols) and red : far-red ratio (closed symbols) in full light (A) and shade (B), at 100 (squares), 262 (diamonds) and 508 (triangles) plants m^{-2}; R : FR *vs.* PAR intercepted (C) for the full light (open symbols) and shade (closed symbols) treatments. Bars indicate ± s.e.

(Table 1 and Fig. 1B). The red : far-red ratio (R : FR) at the soil surface declined as the canopy expanded (Table 1 and Fig. 1A, B). Both in control and shaded canopies, R : FR declined faster with thermal time when the population density was higher. However, significantly smaller values for b (eqn 2) were found only for D1c compared with D3c, and for both D1s and D2s compared with D3s (Table 1). For the relationship between R : FR and P_{int} (Fig. 1C, Table 1), population densities were combined since the separate fits were not significantly different. The difference between fitted lines for the shade and non-shaded treatment was significant for coefficient c ($P < 0.001$).

Dynamics of tiller numbers

Tillering started at a physiological age of approx. 3 phyllochrons (Fig. 2), i.e. around the appearance of the third leaf of the main stem. The shaded plants did not produce any secondary tillers (inset in Fig. 2). In the control plants, grown in full sunlight, progress in tillering was similar for all population densities up to a physiological age of approx. 4·8 phyllochrons, when the plants had approx. three appeared tillers. After this point tiller production diverged as fewer additional tillers appeared at higher population densities. The maximum number of appeared tillers was lower at higher population densities, with more tillers being produced by plants in full light than by shaded plants (8·9, 5·7 and 3·7 tillers for D1c, D2c and D3c, and 3·0, 1·3 and 0·7 tillers for D1s, D2s and D3s, respectively). After reaching a peak, tiller number declined by senescence to 3·8, 1·9 and 0·1 tillers per plant for D1c, D2c and D3c, and to 2·6, 1·0 and 0·1 tillers for D1s, D2s and D3s, respectively. The final number of tillers did not decrease any further beyond the data points shown in Fig. 2. In none of the treatments did tiller senescence occur before the last tiller appeared (data not shown), i.e. there was no overlap between tiller appearance and senescence.

Within the non-shaded and within the shaded plants, population density did not affect the physiological age at which primary tillers appeared (Fig. 3); in D3c, appearance of t4 occurred in only one out of ten plants, and therefore the data point for this tiller does not count as evidence. In the shaded plants, appearance of a tiller type occurred consistently at a slightly higher physiological age than in control plants under full sunlight. The maximum delay in tiller appearance caused by shade was approx. 1 phyllochron for any population density, as observed for t3 in the D1s treatment.

Cessation of visible tiller appearance

For D1, control and shaded plants reached maximum tiller number at the same physiological age (approx. 7·4 phyllochrons, when calculated on an individual plant basis; Fig. 4A). For D2 and D3, physiological age at maximum tiller number was significantly lower for the shaded plants ($P < 0.05$) than for the plants in full light, whose physiological age at maximum tiller number was significantly lower ($P < 0.05$) at higher population densities. In the shaded plants, D2 and D3 had a significantly lower physiological age at maximum tiller number than D1

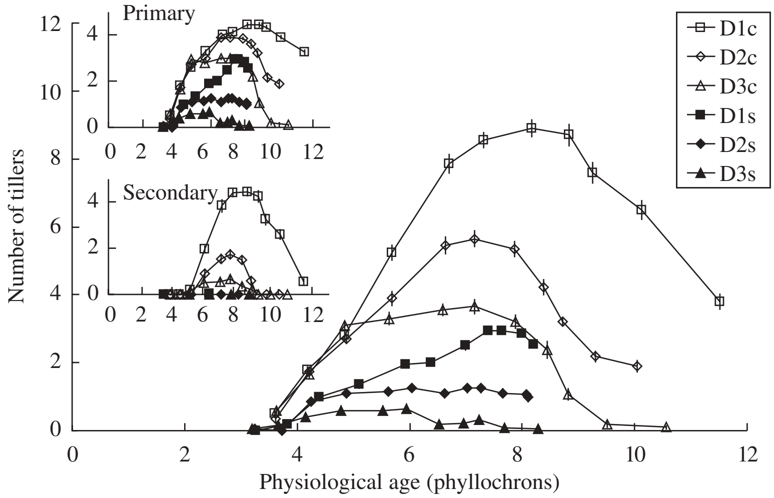

FIG. 2. Relationships between mean number of (non-senescing) tillers and physiological age of the main stem, calculated using leaf appearance data for each population density × light intensity combination, in full light (open symbols) and shade (closed symbols), at 100 (squares), 262 (diamonds) and 508 (triangles) plants m^{-2}. The insets show the same data split into two groups containing primary (above) and secondary (below) tiller data. Vertical bars indicate ± s.e., $n = 120$.

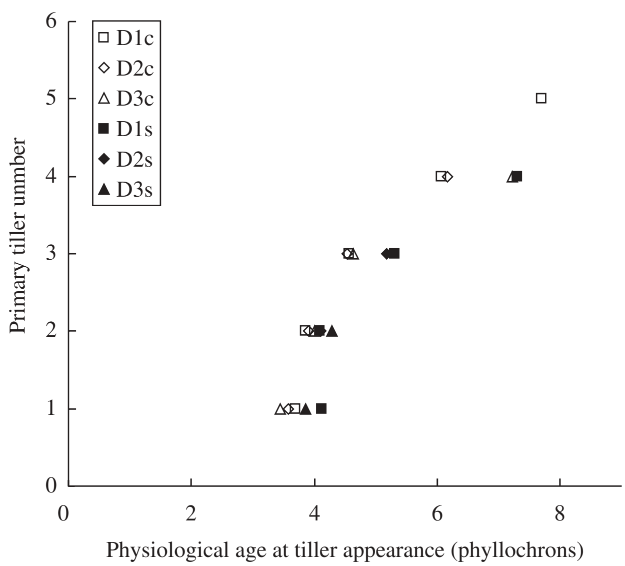

FIG. 3. Relationships between the appearance of primary tillers and the physiological age of the main stem, in full light (open symbols) and shade (closed symbols), at 100 (squares), 262 (diamonds) and 508 (triangles) plants m^{-2}, $n = 60$.

($P < 0{\cdot}05$), but the values for D2s and D3s did not differ significantly from each other.

P_{int} and R : FR (both calculated from the fitted functions; Table 1 and Fig. 1) at maximum tiller number were stable across population densities within the control and shaded treatments (Fig. 4B and C), with the control treatments intercepting 0·53–0·62 PAR and having R : FR ratios of 0·24–0·30 at maximum tiller number. In the shaded plants, treatments D1s and D3s had similar values for P_{int} (0·61 and 0·59, respectively) and for R : FR (0·37 and 0·36 in D1s and D3s, respectively), values that were similar to those of all control plants. However, the D2s treatment intercepted less PAR and had a higher R : FR ratio ($P < 0{\cdot}05$) at maximum tiller number than the D1s and D3s treatments.

Occurrence of different tiller types and arrest of outgrowth of tiller buds

In full sunlight, the probability of occurrence of tillers t1, t2 and t3 was not affected by population density (Table 2), with a probability of 1 in all cases. The probability of occurrence of t4 was 0·8 in D2c and 0·1 in D3c. Tiller t5 appeared only at the lowest population density (probability 0·1). In the shaded plants, increasing population density reduced the probability of occurrence of most tillers, the extent of which depended on tiller position. For D1s, only t2 and t3 were present on all plants and, in D2s, t2 was the only tiller type with a probability of 1·0. The probability of occurrence of t1 under shade was conspicuously low and erratic (0·2, 0 and 0·3 in D1s, D2s and D3s, respectively). Plotting these probabilities against mean P_{int} by the canopy during a window of opportunity for bud outgrowth (starting at ligule appearance of the bud's parent leaf, and ending at ligule appearance of the next leaf), and excluding the t1 probabilities under shade, a clear relationship was found (Fig. 5A). Independent of population density and with little effect of shade, the probability of any primary tiller occurring was 1 until P_{int} reached approx. 0·4. After this point, the probability dropped until it reached 0 at values of P_{int} of approx. 0·55, and remained 0 beyond that value of P_{int}. The only exception was t1 in the shaded plants, for which there was no relationship between P_{int} and probability of occurrence: t1 showed low probability of occurrence when the P_{int} was still considerably lower than 0·4 (Fig. 5A). A similar plot for R : FR shows that a clear distinction was visible between non-shaded and shaded plants (Fig. 5B): the threshold R : FR values below which the probability of occurrence approached zero, were approx. 0·51 for plants grown in shade and 0·32 for control plants.

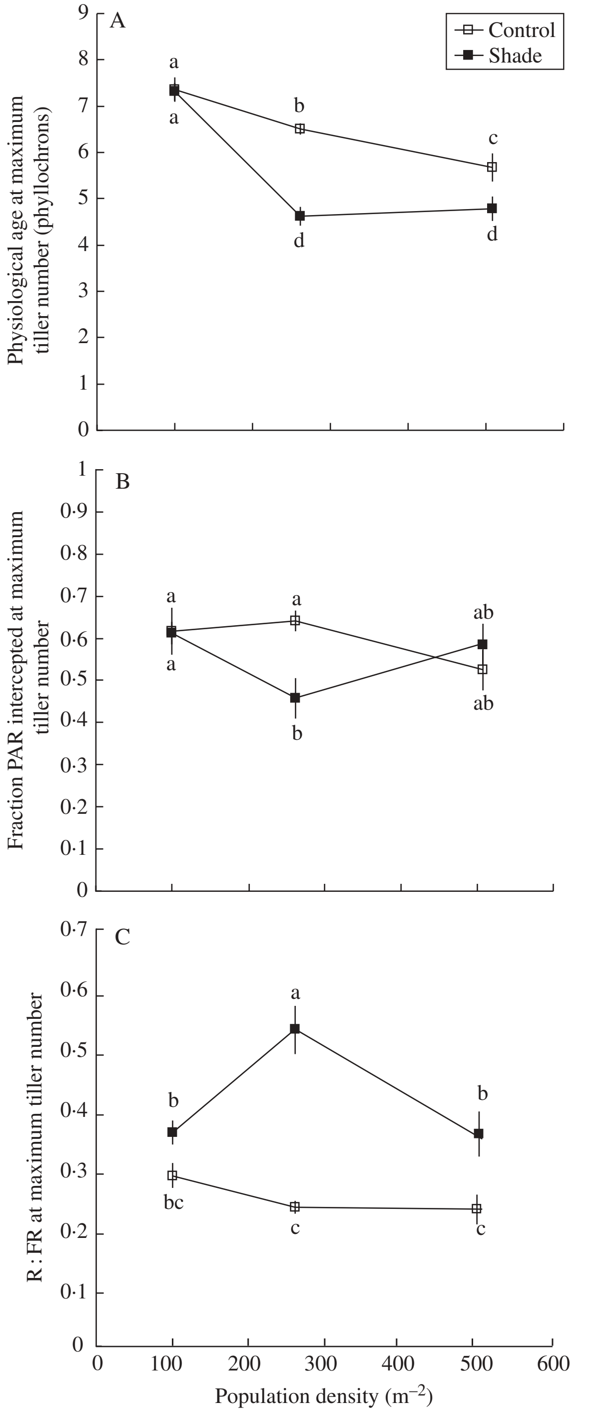

FIG. 4. Relationships between the physiological age of the main stem at maximum tiller number (A) and the population density, and concurrent fraction of PAR intercepted (B) and R : FR (C) in full light (open symbols) and shade (closed symbols). Letters indicate significance group ($P < 0.05$) per variable. Vertical bars indicate ± s.e., $n = 60$.

LMA of parent leaves and outgrowth of buds

Both within the group of buds that had grown out, and the group of buds that remained dormant, there was no significant difference between population densities in

TABLE 2. *Probability of occurrence of primary tillers t1 to t5 for plants at three population densities (D1, D2 and D3, i.e. 100, 262 and 508 plants m^{-2}) and two light regimes (c = control, s = shade)*

Tiller	D1c	D2c	D3c	D1s	D2s	D3s
t1	1·0	1·0	1·0	0·2	0	0·3
t2	1·0	1·0	1·0	1·0	1·0	0·5
t3	1·0	1·0	1·0	1·0	0·2	0
t4	1·0	0·9	0·1	0·9	0	0
t5	0·3	0	0	0	0	0

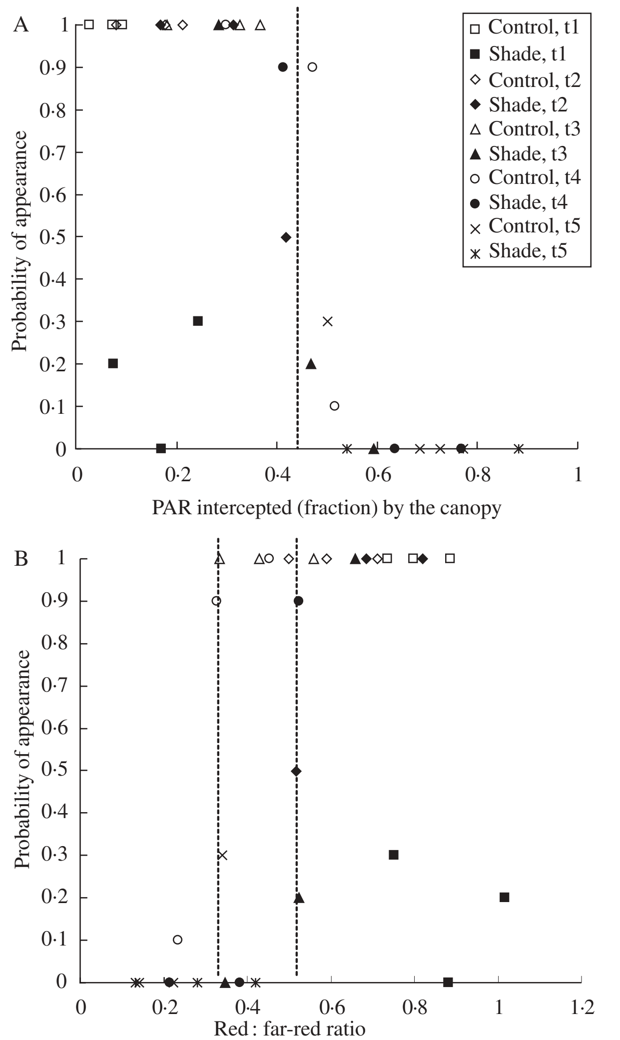

FIG. 5. Relationships between probability of occurrence of tillers t1 to t5 and the fraction of PAR intercepted (A) and R : FR (B) by the canopy, during a window of opportunity for bud outgrowth starting at ligule appearance of the parent leaf and ending at ligule appearance of the next leaf. The vertical dashed lines show the threshold levels at which probabilities of occurrence decrease. Population densities are not differentiated, $n = 60$. Note that in (B) the course of R : FR over thermal time should be read from right to left.

LMA of the parent leaf blades. LMA of the parent leaves of buds of t1 to t5 in the 'dormant group' (all population densities combined) was 2·78 mg cm^{-2} [standard error (s.e.) = 0·09, $n = 40$], in the 'outgrowth group' LMA was

3·86 mg cm^{-2} (s.e. = 0·13, n = 42), a highly significant (P < 0·001) difference. The probability distribution of LMA data for the 'dormant' and 'outgrowth' groups (Fig. 6) indicates that the class limit beyond which the tillers of the outgrowth group had a higher probability of appearing than those of the dormant group (dashed line in Fig. 6) was 3·75 mg cm^{-2}. The probability of dormancy of buds for which the parent leaf's LMA was lower than 3·75 mg cm^{-2} was 0·63. For LMA >3·75 mg cm^{-2}, the probability of outgrowth was 0·84. Separate analysis on data from phytomer number four showed the greatest variation among population densities in probability of occurrence of tiller appearance (Table 2), resulting in a balanced distribution between the 'outgrowth' and 'dormant' groups with n = 8 and n = 11, respectively. A significant difference (P < 0·01) between the two groups was found, i.e. LMA was 3·99 (s.e. = 0·30) mg cm^{-2} for the 'outgrowth group' and 1·99 (s.e. = 0·16) mg cm^{-2} for the 'dormant' group. This difference was primarily caused by longer and heavier leaves in the 'outgrowth' group compared with the 'dormant' group. The frequency distribution indicated a threshold LMA value of 3·25 mg cm^{-2} (not shown), with an outgrowth probability of 0·83 when the LMA of the parent leaf was above the threshold, and a dormancy probability of 0·77 when it was below the threshold.

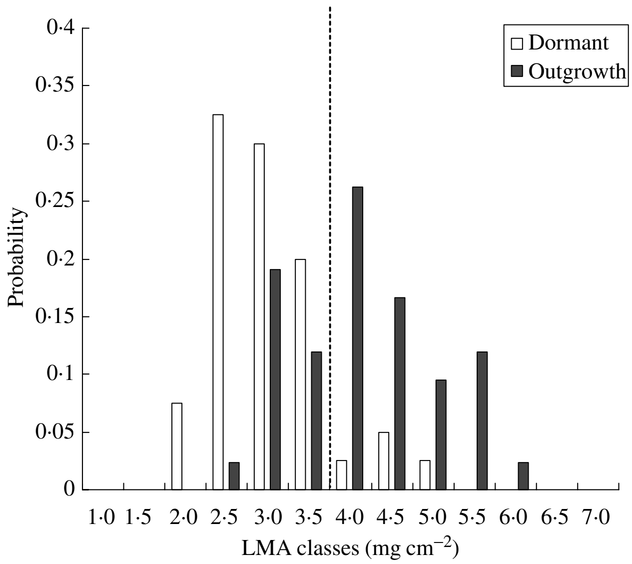

FIG. 6. Probability distribution of leaf mass per unit leaf area (LMA) of parent leaves of buds t1 to t5 that either remained dormant ('dormant' group, open bars) or grew out ('outgrowth' group, closed bars), of buds 1–5 on the main stem, n = 82. The dashed line indicates the class limit below which the distribution for the dormant group has a higher probability, and above which the outgrowth group has a higher probability.

DISCUSSION

Tiller numbers

In full light, population density had no effect on total number of tillers per plant until approx. 4·8 phyllochrons had been produced (Fig. 2) but, from that point on, tillering rates started to diverge between population densities. A similar divergence in tillering rate in relation to population density has been observed in pearl millet (van Oosterom *et al.*, 2001) and sorghum (Lafarge and Hammer, 2002). In the current study, this divergence can be attributed partly to the appearance of more secondary tillers (inset in Fig. 2) at lower population densities. Furthermore, at the divergence, primary tiller number (inset in Fig. 2) in D3c plants reached a plateau of approx. three tillers, a value that was maintained for 3 phyllochrons. This plateau was caused by constant tiller numbers, and not by compensation between appearance and senescence of tillers, as senescence occurred later; the first tillers started to senesce from a physiological age of 7·1 phyllochrons onwards, and the senescence of later tillers did not start till a physiological age of 8 (data not shown). In D1c and D2c, the plateau in tiller numbers was either not present or much less distinct than in D3c (inset in Fig. 2) due to the higher probability of occurrence (Table 2) of t4 and t5 at those population densities. Similar effects of population density on tiller appearance of t2 to t5 in spring wheat have been reported by Bos (1999) (note: t1 was absent in his experiment), while Kirby and Faris (1972) showed that development of barley tiller buds up to t4 was not affected by population density, but that subsequent buds appeared later at higher population densities due to the delaying effect of population density on developmental rate of the plant.

Cessation of tillering in relation to fraction of PAR intercepted

The emergence of new tillers has been found to decrease strongly at a particular leaf area index (Simon and Lemaire, 1987; Lafarge and Hammer, 2002). The current analysis (Fig. 4B) indicates that, in full sunlight, tiller appearance stopped at a particular value of P_{int} (0·59 on average) rather than at a fixed physiological age. In shade treatments, the appearance of tillers in D1s and D3s ceased at approximately the same P_{int} as in full sunlight. However, in D2s, cessation of tillering occurred at lower P_{int} than in D1s and D3s (0·45, P < 0·05). Omitting the case of D2s results in a mean P_{int} of 0·60 in the shaded plants, a value similar to that of the plants in full light (0·59). The data shown in Fig. 5 are independent of Fig. 4, which relates cessation of appearance of leaves to conditions in the canopy. However, the 'decision' of a tiller bud to remain dormant instead of growing out must have been taken approx. 1 phyllochron before the time of maximum tiller number, because the period of time during which the bud extends within the surrounding sheath has to be taken into account. The analysis of Fig. 5 refers to that period. Therefore, these results reinforce the proposition that tiller appearance ceased at a similar P_{int} in full sunlight and in shaded plants. On the basis of the data in Fig. 5A, it may be surmised that the probability of a tiller to grow out starts to decrease when P_{int} is >0·40, and that it has fallen to zero by the time that P_{int} exceeds 0·55. This translates to a time window of around 0·75 phyllochrons for the fully lit plants, and

0·85 phyllochrons for the shaded plants. In other words, well within the time it took for two successive leaves to appear, complete tillering cessation had taken place.

LMA of parent leaves and tiller outgrowth

Bos (1999) hypothesized that low PAR intensity at the level of the a bud's parent leaf, and therefore its low rate of assimilate supply, prevents the bud from developing into a tiller. Bos (1999) reported a relationship between the mean LMA of a particular leaf rank and the probability of occurrence of the tiller. He found a 'threshold' value for LMA of 2·9–3·1 mg cm^{-2} for t1 and 2·0 to 2·4 mg cm^{-2} for t3, below which buds did not grow out and above which they did. These results appear to be consistent with the present results, but there are some important differences. In the current study the parent leaf is regarded to be the leaf on the same phytomer, being the leaf directly above the tiller bud (Evers *et al*., 2005) and not the leaf in the axil of which the bud resides as in Bos (1999). The current interpretation of the association between parent leaf and bud is preferred to that of Bos because it has been shown for barley that an initiated bud is nutritionally dependent on the leaf above the bud rather than the subtending leaf. This view is based on anatomical grounds and on the relationship between the photosynthetic activity of that leaf and bud growth (Fletcher and Dale, 1974). Analysis of the relationship between tiller appearance and LMA of the subtending leaf, as was done by Bos (1999), yielded largely the same segregation in LMA distributions for parent leaves of dormant buds than of buds that grew out, but less distinctively to that found for the parent leaves (as defined here). Yet, if bud growth is directly or indirectly associated with LMA of leaves, it is not only the LMA of the parent leaf, but it may also be LMA of the leaf on the preceding phytomer (the subtending leaf) that counts. The LMA threshold values for arrest of tiller growth in Bos (1999) were lower than that of the present study (3·75 mg cm^{-2}), a result which may be explained by the difference in growing conditions (growth chamber lamps *vs*. full sunlight). The current investigation advances understanding because the association between LMA and bud growth was evaluated on an individual plant basis, resulting in probabilities of bud outgrowth in relation to LMA values (Fig. 6) rather than population means reported by Bos (1999).

The red : far-red ratio and cessation of tillering

It has been argued that, in ryegrass (Simon and Lemaire, 1987) and sorghum (Lafarge and Hammer, 2002), cessation of tillering can be explained by photomorphogenetic effects particularly changes in R : FR (e.g. Casal *et al*., 1990), rather than reduction in the rate of photosynthesis per (parent) leaf, since no association could be found between availability of assimilates in the tiller bases and cessation of tillering in the case of rye grass (Davies and Thomas, 1983). Such photomorphogenetic effects on tillering are mediated mainly by R : FR of horizontally propagated light perceived by vertically orientated organs such as elongating leaves, sheaths of mature leaves, and internodes (Cordukes and Fisher, 1974; Morgan *et al*., 1980; Skinner and Simmons, 1993). Next to the R : FR ratio, the supply of blue light has also been shown to play a role in modulation of tillering (Barnes and Bugbee, 1992). R : FR is closely related to P_{int} (Fig. 1C), because light 'emitted' (reflected and transmitted) by the surrounding vegetation is strongly depleted in the red region of the spectrum compared with the far-red region (Holmes and Smith, 1977), but this is not true for direct light. At the time of maximum number of tillers, horizontally measured R : FR at soil level was 0·26 in full light, irrespective of population density (Fig. 4C), whereas physiological age varied significantly among population densities. In the shaded plants, D2s had a significantly higher R : FR value (0·54, $P < 0{\cdot}001$) at the time of cessation of tiller appearance than D1s and D3s which had an identical average R : FR of 0·36. Therefore, in full light conditions, an R : FR of approx. 0·25 to 0·3 represented a 'threshold' value above which (at least indirectly) tiller appearance ceased. In shaded conditions, the value may be higher, due to the difference in the relationship between P_{int} and R : FR (Fig. 1C). When taking into account the time it takes for a tiller to appear from its surrounding sheath, the R : FR threshold value for cessation of outgrowth was 0·35–0·40 in full light, and 0·45–0·50 in shade (Fig. 5B). However, the former value is much more representative of agricultural systems than the latter, since 75 % shading does not occur in the field.

Thresholds in P_{int}, R : FR and LMA of parent leaves: different sides of the same coin?

A low R : FR results in lower LMA values in chrysanthemum (Rajapakse and Kelly, 1992) and tall fescue (Wherley *et al*., 2005). Wherley *et al*. (2005) hypothesized that the decrease in tillering in their low R : FR treatment was due to the decreased LMA and the associated decrease in photosynthetic capacity of the leaves. The existence of a association between LMA and photosynthetic capacity was argued by Oguchi *et al*. (2003), although in some cases no association has been found over a substantial range of LMA values (Vos and van der Putten, 1998). A general reduction in LMA with increase in population density was not found in the current study, but has been described for potato (Vos, 1995) and ryegrass (van Loo, 1993). van Loo ascribed the effects of a high population density on LMA to reduced light intensity (Silsbury, 1971). However, critical evidence supporting that mechanism seems to be lacking; there are no data showing divergence in LMA of (full grown) leaves that initially showed the same LMA but diverged in LMA after being subjected to high and low light regimes, respectively. Most evidence suggests an environmental trigger (i.e. R : FR), which results in a morphogenetic change in LMA. If variation in LMA of leaves of a particular phytomer rank is brought about by a photomorphogenetic response, this variation in LMA reflects the range of light conditions experienced by these leaves and also underlines the local nature of the response: the light conditions (R : FR) of each parent leaf × bud combination are apparently decisive for the LMA of the parent leaves and outgrowth of the tiller bud.

Several authors have proposed a relationship between tiller outgrowth and substrate production per plant or per parent leaf (e.g. Bos and Neuteboom, 1998; Gautier *et al.*, 1999). Obviously, bud growth requires substrates, but the question is whether or not substrate availability is the main determinant of the fate of a tiller bud. If it were, one would not expect a conservative value across population densities and shading for the P_{int} that marks the cessation of tiller appearance. Therefore, it is concluded, in this investigation, that light quality dominated over substrate availability as a determinant of the fate of tiller buds.

Nevertheless, the differences in probability of occurrence of t1 in the shaded plants between population densities (Table 2, Fig. 5) could be a direct result of the reduced amount of PAR on (parent) leaf 1, rather than being triggered by P_{int} or the value of R : FR (Fig. 5A, B). At the early stage of development of the canopy, at which the window of opportunity for t1 to grow out has opened, R : FR is still high but the absolute amount of incident PAR is low in the shaded plants. Under those conditions the small size of leaf 1 may limit the resources provided by this leaf for the t1 bud to grow out, resulting in much reduced occurrence of t1 in shade as compared with those in full sunlight.

In conclusion, the current results, along with evidence from the literature, support the theory that tillering ceases at a fixed R : FR and P_{int} inside the canopy. Particularly in the case of fully sunlit plants, the time of maximum tiller number occurred at a similar P_{int} and at a similar red : far-red ratio, independent of population density, in spite of the fact that plant development, expressed in physiological age, was significantly different between population densities. This suggests that particular light conditions, related to canopy development stage, suppress bud outgrowth. During the window of opportunity for outgrowth of buds, it appears that a value of 0·40–0·45 for P_{int} marked the transition between bud growth (P_{int} < 0·40–0·45) and suppression of bud growth (P_{int} > 0·40–0·45). Furthermore, for plants grown in full light, bud outgrowth was suppressed when R : FR dropped below a threshold value (0·35–0·40). The population density used in the field usually does not exceed the range of population densities used in this trial, and the light intensity caused by the shade treatment is far below natural light intensity. Therefore, the main conclusions drawn from treatments exposed to full sunlight are probably valid for common agronomic conditions for wheat growth, provided that water and N are applied in non-limiting amounts.

ACKNOWLEDGEMENTS

The authors thank Peter van der Putten, Ans Hofman, Henriette Drenth and Unifarm for their contributions in the experiment, Peter Hochs for valuable help in the development of the blade shape function, and Dr G. Russell and an anonymous reviewer for helpful comments on the manuscript. The C. T. de Wit Graduate School for Production Ecology and Resource Conservation funded this work; the Netherlands Organisation for Scientific Research (NWO) provided additional support.

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APPENDIX A

A function (eqn A1) was developed that describes the shape of the full-grown wheat blade by calculating the distance from blade margin to midrib as a function of distance to the blade tip:

$$W_{\mathrm{norm}} = \left(\frac{-L_{\mathrm{norm}}(L_{\mathrm{norm}} - 2L_{\mathrm{m}})}{L_{\mathrm{m}}^2}\right)^C \qquad (A1)$$

For derivation of this function, seven to ten measurements of the distance of the blade margin to the midrib were made on eight randomly chosen fully grown spring wheat leaves. Flag leaves were excluded because their shape diverges slightly from that of the other leaves. W_{norm} is the normalized distance between margin and midrib (actual divided by final maximum margin–midrib distance), L_{norm} is the normalized distance of the leaf base to the leaf tip (distance to leaf tip divided by final maximum blade length), L_{m} is the distance of the point of maximum margin–midrib distance to the blade tip as a fraction of the final length ($0{\cdot}5 < L_{\mathrm{m}} < 1$), and C is a curvature coefficient ($0 < C < 1$). For the spring wheat cultivar used in this study, coefficient values were $L_{\mathrm{m}} = 0{\cdot}603$ (s.e. = 0·0047) and $C = 0{\cdot}63$ (s.e. = 0·015) ($R^2 = 0{\cdot}97$, $n = 68$).